The evolution of apical dominance in maize

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The domestication of crop plants has often involved an increase in apical dominance (the concentration of resources in the main stem of the plant and a corresponding suppression of axillary branches)[1]. A striking example of this phenomenon is seen in maize (*Zea mays* spp. *mays*), which exhibits a profound increase in apical dominance compared with its probable wild ancestor, teosinte (*Zea mays* ssp. *parviglumis*)[2]. Previous research has identified the *teosinte branched1* (*tb1*) gene as a major contributor to this evolutionary change in maize[3]. We have cloned *tb1* by transposon tagging and show here that it encodes a protein with homology to the *cycloidea* gene of snapdragon[4]. The pattern of *tb1* expression and the morphology of *tb1* mutant plants suggest that *tb1* acts both to repress the growth of axillary organs and to enable the formation of female inflorescences. The maize allele of *tb1* is expressed at twice the level of the teosinte allele, suggesting that gene regulatory changes underlie the evolutionary divergence of maize from teosinte.

Teosinte plants typically bear an elongated lateral branch at most nodes on their main stems[2]. These branches are tipped by male inflorescences (tassels) and the slender female inflorescences (ears) are borne on secondary branches in the axils of the leaves on the primary branches. In contrast, maize plants typically produce a lateral branch at only two or three of the nodes on their main stems, and these are short and tipped by ears. Previously, we demonstrated that these differences in plant architecture are governed by a small number of quantitative trait loci (QTL)[5]. One of these QTL was shown by genetic complementation testing to correspond to the

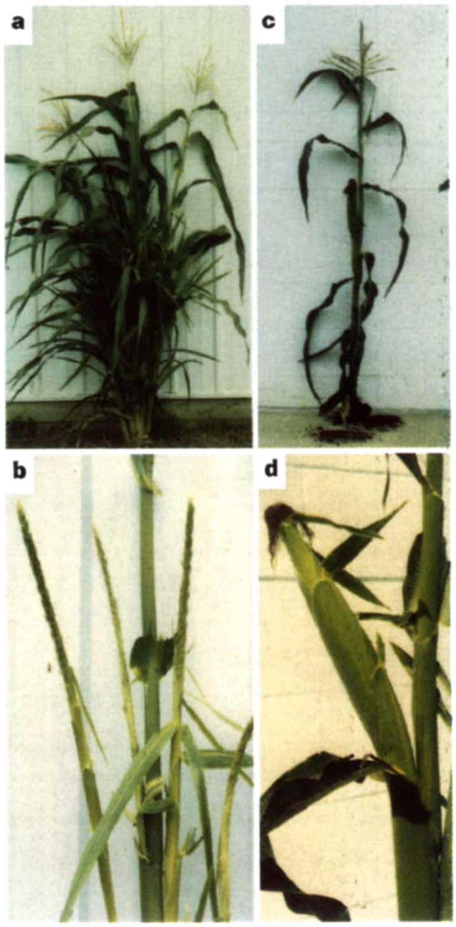
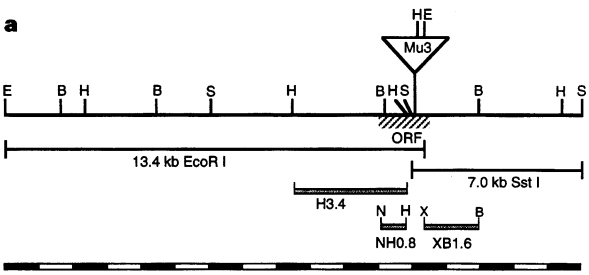


Figure 1 Plants and inflorescences of wild-type and *tb1-ref* mutant maize. **a**, **b**, *teosinte branched1* mutant. **c**, **d**, Wild-type maize (Inbred A158).

in our population for the degree of tiller growth, the number of ear



b

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     L  G  V   P  S  V   K  H  M  F   P  F  C   D  S  S   S  P  M  D   L  P  L   Y  Q  Q
  1  GCCTTGGAGT CCCATCAGTA AAGCACATGT TTCCTTTCTG TGATTCCTCA AGCCCCATGG ACTTACCGCT TTACCAACAA
     L  Q  L  S   P  S  S   P  K  T   D  Q  S  S   S  F  Y   C  Y  P   C  S  P  P   F  A  A
 81  CTGCAGCTAA GCCCGTCTTC CCCAAAGACG GACCAATCCA GCAGCTTCTA CTGCTACCCA TGCTCCCCTC CCTTCGCCGC
     A  D  A   S  F  P  L   S  Y  Q   I  G  S   A  A  A  A   D  A  T   P  P  Q   A  V  I  N
161  CGCCGACGCC AGCTTTCCCC TCAGCTACCA GATCGGTAGT GCCGCGGCCG CCGACGCCAC CCCTCCACAA GCCGTGATCA
     S  P  D   L  P  V   Q  A  L  M   D  H  A   P  A  P   A  T  E  L   G  A  C   A  S  G
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Figure 2 Structure of *teosinte branched1*. **a**, Restriction endonuclease maps of overlapping genomic λ clones (E13.4 and S7.0) of *tb1*. Insertion site of the *Mu3* element in the *tb1* open reading frame (ORF, hatched box) is shown. Three probes (H3.4, NH0.8 and XB1.6) used in Southern and northern blot analyses are shown. Abbreviations for restriction enzymes: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; N, *Nco*II; S, *Sst*I; and X, *Xba*I. Not all N and X sites were mapped. Scale in 1 kb segments. **b**, Nucleic-acid and deduced amino-acid sequence of the *tb1* cDNA clone. Two domains conserved between *tb1*, *cycloidea* and *Arabidopsis* ESTs are highlighted. Putative bipartite nuclear localization signal is underlined. Sequences duplicated by the *Mu* insertions are double underlined.

Table 1 Morphometric comparison of mutant and wild-type alleles of *tb1*

Genotypes

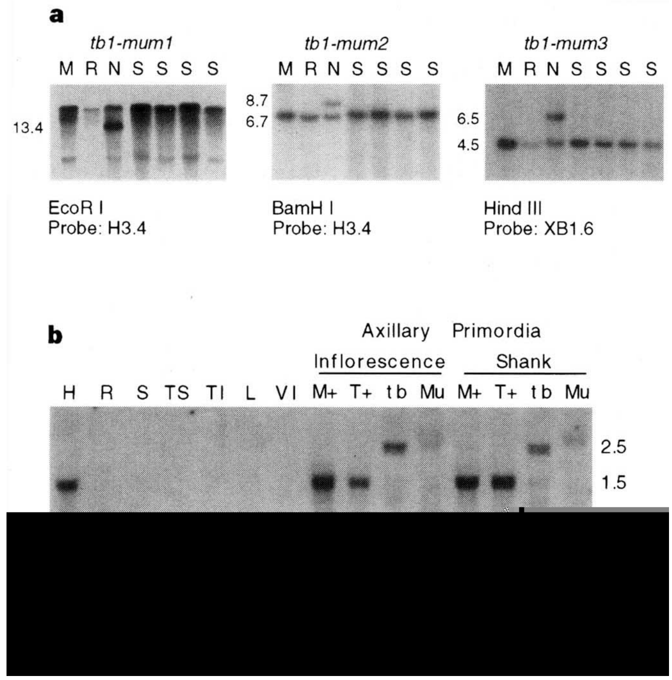

Figure 3 a, Southern blot analyses. *tb1-mum* alleles each contain an insertion in *tb1* as compared to the parental *tb1-ref* and *Mu* stocks, and 20 (4 shown) full sibs of each mutant plant. The *Mu3* element in the *tb-mum1* allele possesses an *Eco*RI site, so this insertion created the 13.4 kb *Eco*RI fragment. The *Mu* insertions in the other two *tb1-mum* alleles increased the sizes of existing fragments by about 2 kb. Abbreviations: *Mu* stock, M; new *Mu* mutant plant from the tagging population, N; *tb1-ref* stock, R; full sibs of the new *Mu* mutant plant, S. **b**, Northern blot analyses. RNAs from inbred W22: ear husk, H; root, R; dark grown seedlings, S; immature tassel spikelets, TS; immature tassel rachis internodes, TI; immature leaves, L; and immature vegetative internodes of the main stem, VI. Additional RNAs from axillary inflorescence and shank primordia of plants homozygous for alleles: *Tb1 +Maize*, M+; *Tb1 +teosinte*, T+; *tb-ref*, tb; *tb1-mum1*, Mu.

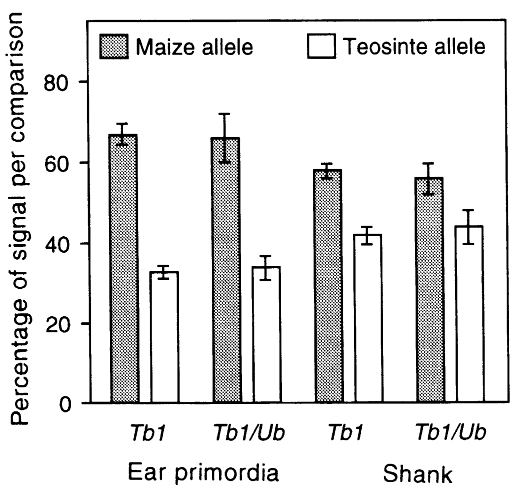

Figure 4 *tb1* message accumulation for ear primordia and immature shank of the maize (*Tb1 +Maize*) and teosinte (*Tb1 +teosinte*) alleles in inbred W22 genetic background. Data are presented both as the amount of *Tb1* message per 10 μg of total cellular RNA and as the amount relative to *Ubiquitin* (*Ub*) message level as determined by phosphor imager readings. Probability (*P*) under the null hypothesis of no difference in message level of the maize and teosinte alleles was less than 0.01 in each case except for *Tb1/Ub* for shank ($P < 0.05$); standard error bars as shown.

ear where *tb1* is expressed, growth of the lodicules (petals) and stamens is arrested.

A previous proposal[3] that *tb1* acts as a repressor of axillary meristem growth is consistent with our expression data in that the axillary organs in which *tb1* is expressed are typically reduced in size. For example, the husks of wild-type maize have reduced leaf blades and the axillary branch internodes of wild-type maize are shorter. Thus, *tb1* may act as a repressor of the growth of those organs in which the gene is expressed. *tb1* also regulates the sex of the inflorescences terminating the lateral branches and is required for the normal formation of ears. This may indicate that *tb1* has functions in addition to that of repressing organ growth.

Previously, we proposed that maize and teosinte would both carry functional alleles of *tb1* but ones whose expression is differentially regulated[3]. A higher level of expression for the maize allele is expected in correspondence with its shorter (more repressed) maize evolution is proposed[3]. In teosinte, *tb1* is functional and is normally expressed in the secondary axillary meristems where it controls their conversion into ear shoots (including the small ear, the single husk that surrounds this ear and the single short internode that subtends it). *tb1* is not normally expressed in the primary axillary meristems of teosinte so that these are able to develop into elongated tassel-tipped branches. During the domestication of maize, humans selected an allelic variant of *tb1* that is expressed in primary axillary meristems (and probably has a high level of expression) such that these form ear shoots rather than elongated tassel-tipped branches. Thus, evolution has not proceeded by a loss/gain or change in *tb1* function, but by an alteration in gene regulation. Consistent with this view, a preliminary comparison of partial amino-acid sequences of maize and teosinte alleles revealed no fixed amino-acid differences between them, suggesting that a change in protein function has not occurred (see Suppledominance relative to their wild ancestors[1]. Comparative QTL mapping[12] suggests that this is not an unlikely prospect. □

Methods

Plant materials. The W22 line carrying *Tbl+ teosinte* was constructed as previously described[3] except with six generations of backcrossing. The *tb1-ref* stock was provided by C. Burnham and the *Mu* stocks by V. Chandler. The *Mu* tagging population, the F_2 population segregating for *tb1-ref*, and plants for quantification of *tb1* message levels were grown at the University of Minnesota Agricultural Experiment Station in St Paul during the summers of 1994, 1995 and 1996, respectively.

Nucleic acid analysis. DNA extraction and Southern hybridizations were done as previously described[5]. Molecular marker loci closely flanking *tb1* were bcd1072, npi615, umc107 and umc140. Genomic DNA restriction fragments carrying the cosegregating *Mu3* element were cloned into either λ Dash or λ

constructed in λ ZAP-Express (Stratagene) from RNA isolated from immature

A common precursor for primitive erythropoiesis and definitive haematopoiesis

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The generation of blood cells, haematopoiesis, in the mouse